Switch-Based Mechanism of Kinesin

Motor-Enzyme Action Captured in "Snapshots" of Two Key States

Without infrastructure, business grinds to a halt, and the business of a cell is no exception. Cells require an internal transportation system reliable and flexible enough to accommodate both the routine movement of organelles and the dramatic choreography of mitosis. In response, nature has engineered an intracellular rail system of sorts, in which a "motor" enzyme called kinesin hauls chromosomes and other cellular freight along microtubule tracks. Disruption of this system can lead to certain neurological disorders as well as cancer. To better understand how this system works, a team of researchers from the University of Tokyo and the University of California, San Francisco, working at the ALS and Stanford Synchrotron Radiation Laboratory, compared the structures of the kinesin mechanism when crystallized in two functionally critical states.

Kinesin's kinetic energy comes from the energy released when adenosine triphosphate (ATP) is hydrolyzed to produce adenosine diphosphate (ADP). The energy is thought to drive a stepping sequence in the kinesin molecule, which, in its conventional dimeric form, has a two-headed bilateral symmetry: one "head" remains attached to the microtubule surface while the other is free to move forward. However, the core region of a monomeric form of kinesin (KIF1A) has been observed to take multiple steps before detaching, suggesting that the KIF1A core region plays a vital role in kinesin's mobility.

In this work, the researchers determined the structure of the KIF1A core region bound to ADP (to 2.2 Å) and to a nonhydrolyzable analogue of ATP (to 2.0 Å). Previously, all attempts to crystallize the ATP-like complex had failed. The research team's success in crystallizing this complex provided an excellent opportunity to compare "snapshots" of two key moments in the kinesin stepping sequence. Although the two structures look very similar overall, marked differences were observed in two "switch" regions near the ATP/ADP binding site.

In the switch I region, a helix flanked by two short loops in the ADP state forms a short β-hairpin structure in the ATP-like state. The switch II region shows a series of structural changes, including the partial unwinding of a helix and its rotation by roughly 20 degrees in the ATP-like state. To visualize the effect of these changes when the kinesin is attached to a microtubule, the researchers embedded the molecular structures within cryo-electron microscope (cryo-EM) images of kinesin in contact with a microtubule surface. The results indicate that the switch II region actually remains fixed relative to the microtubule, while the rest of the KIF1A core rotates by about 20 degrees in the opposite direction. The authors suggest that this rotation binds the kinesin more tightly to the microtubule and creates a directional bias by pointing the tip of the KIF1A core in the direction of motion.

Another important difference between the two states was found in the "neck linker" domain, which connects the two heads in the dimeric form of kinesin (and which was "grafted" onto the KIF1A monomer for this experiment). The structures show that the linker is "docked" near the core in the ATP-like state but is undocked and disordered in the ADP state. This finding supports the hypothesis that the neck linker is a crucial part of the mechanism that drives kinesin. In this view, the kinesin core is a modular base onto which different types of neck domains serve as mechanical amplifiers and transmitters (transmissions and drive shafts) whose exact function depends on the kinesin variant to which the neck linker belongs.

In general, these results confirm expectations—arrived at by analogy to previously studied, structurally similar proteins—that the conformational changes observed in KIF1A are modular and extend to all kinesins. They also suggest a rationale for kinesin's tendency to move in a given direction along a microtubule. ■

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M. Kikkawa, E.P. Sablin, Y. Okada, H. Yajima, R.J. Fletterick, N. Hirokawa, "Switch-based mechnanism of kinesin motors," *Nature* **411** (2001) 439.

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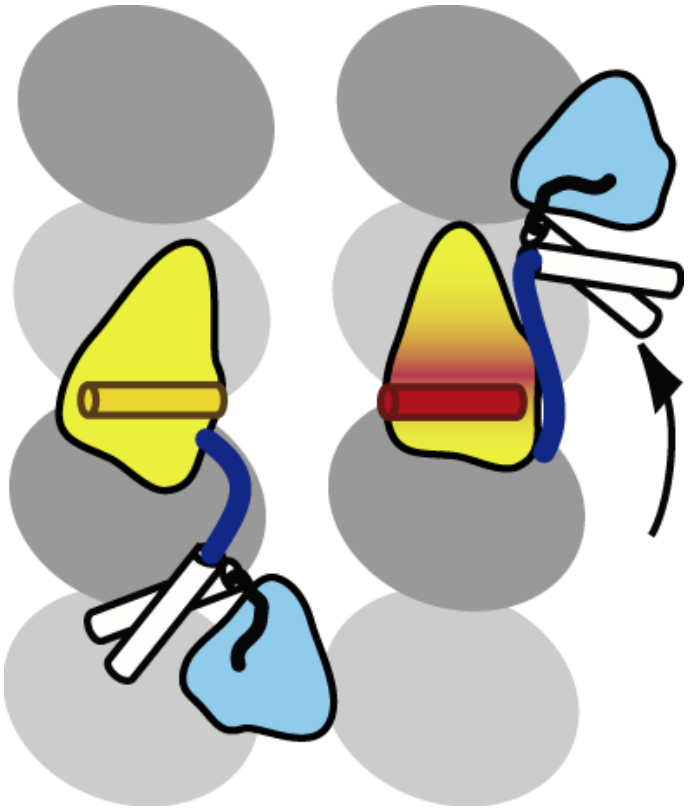
- Kinesin "walks" along microtubule tracks
  - *ATP-to-ADP conversion supplies energy*
  - *Carries neurotransmitters to axon extremities*
  - *Propels chromosomes apart during cell division*
  - *Movement of other organelles within cell*
- Breakdowns lead to serious diseases
  - *Neuronal cell death: neurological disorders*
  - *Runaway cell division: cancer*
- Compared structures in two key states
  - *Bound to ADP (2.2 Å), ATP-like analogue (2.0 Å)*
  - *First successful crystallization of ATP-like analogue*
  - *Results combined with cryo-EM images*
- Structural changes confirm expectations
  - *Modular, extend to all kinesins*
  - *Similar to those in related enzymes*
  - *Provide possible explanation for directional bias*



ADP **ATP-like**

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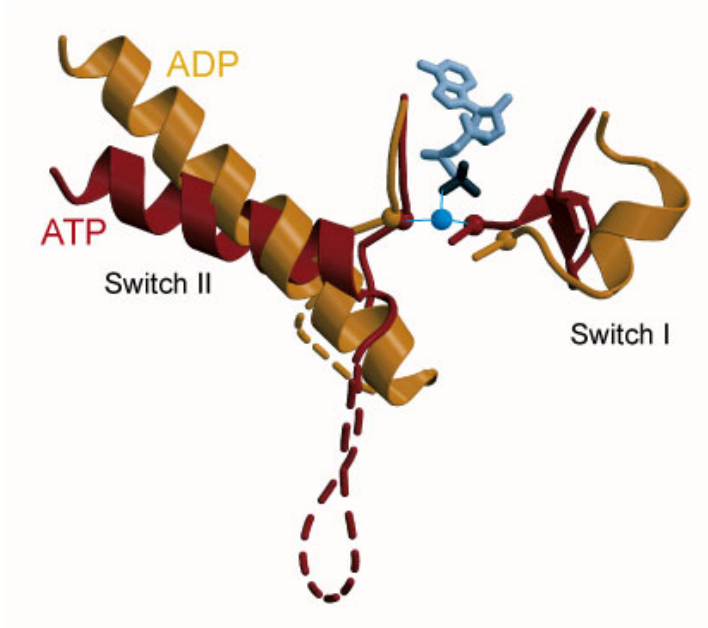

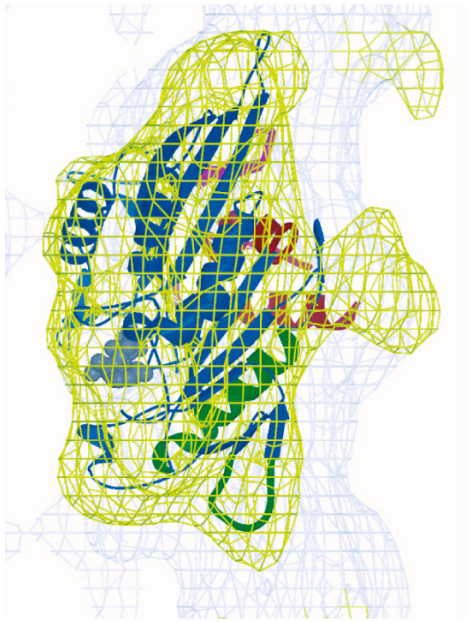

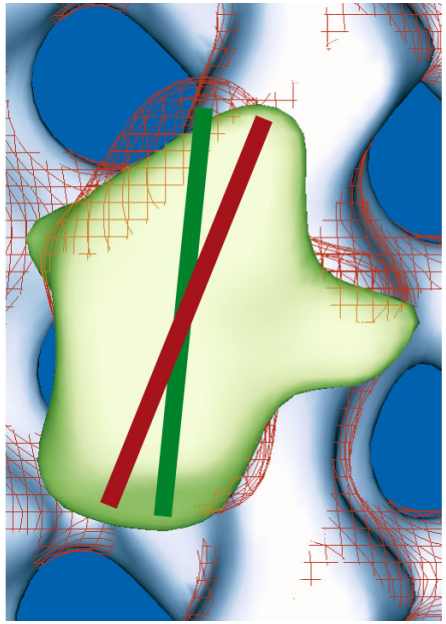

*Comparison of KIF1A switch regions in the ADP **(gold)** and ATP-like **(red)** states. Part of the bound ATP is shown in gray.*

*Rotation of KIF1A core relative to microtubule surface. Left: Kinesin molecular model embedded within a cryo-EM map of kinesin. Right: Kinesin orientation in ADP **(green)** and ATP-like **(red line)** states.*

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